

WHAT IS CLAIMED IS:

1. A chimeric protein comprising:

a) a plurality of zinc finger domains; and

b) a heterologous protein transduction domain, effective for

translocating the protein across a cellular membrane.

2. The chimeric protein of claim 1, wherein the protein transduction domain is N-terminal or C-terminal to the plurality of zinc finger domains.

3. The chimeric protein of claim 1, wherein the plurality of zinc finger domains comprises 3 to 6 zinc finger domains.

4. The chimeric protein of claim 1, wherein the chimeric protein specifically binds to a site in the VEGF-A gene and can regulate transcription of the VEGF-A gene in a cell.

5. The chimeric protein of claim 1, wherein the chimeric protein specifically binds to a site in a gene and can regulate transcription of the gene in a cell, the gene encoding a protein selected from the group consisting of: jun B proto-oncogene, protein kinase C, lectin, brain-specific Na-dependent inorganic phosphate cotransporter, cellular retinoic acid-binding protein 1, cellular retinoic acid-binding protein 2, cadherin 13, H-cadherin (heart), vascular endothelial growth factor (VEGF-A), pigment epithelium-derived factor (PEDF), differentiation-related gene-1 (Drg-1), Transcription factor E2F, Early growth response-1 (EGR-1), protein tyrosine phosphatases 1B (PTP-1B), A20, Fas, melanoma differentiation associated gene-7 (MDA-7), presenilin-1 (PS-1), angiotensin converting enzyme, Angiopoietin-2, b-secretase(BACE1), mmp3, checkpoint with forkhead associated and ring finger (CHFR), peroxisome proliferator-activated receptor gamma (PPAR-gamma), TNF-related apoptosis-inducing ligand (TRAIL), Ku-80, ataxia-telangiectasia mutated (ATM), BRCA, CC-chemokine receptor 5 (CCR5), brain-derived neurotrophic factor (BDNF), tumor necrosis factor alpha-induced protein-3 (TNFAIP3) (A20), c-myc, Hypoxia-inducible factor-1 alpha (HIF-1alpha), caspase-3, intercellular adhesion molecule type I (ICAM-1), angiotensin II receptor 1 (AT-1R), platelet-derived growth factor, insulin-like growth factor-I

and -II, nerve growth factor, aFGF, bFGF, epidermal growth factor, TGF- α , TGF- β , erythropoietin, thrombopoietin, mucins, growth hormone, proinsulin, insulin A-chain, insulin B-chain, parathyroid hormone, thyroid stimulating hormone, thyroxine, follicle stimulating hormone, calcitonin, factor VIII, hematopoietic growth factor, enkephalinase, Mullerian-inhibiting substance, gonadotropin-associated peptide, tissue factor protein, inhibin, activin, interferon- α , interferon- β , interferon- γ , M-CSF, GM-CSF, G-CSF, IL-1, IL-2, IL-3, IL-4, IL-12, and IL-13.

6. The chimeric protein of claim 1, wherein the plurality of zinc finger domains includes domains whose DNA contacting residues correspond to DNA-contacting residues of a set of motifs in a row of Table 2.

7. The chimeric protein of claim 1, wherein the plurality of zinc finger domains includes domains whose DNA contacting residues correspond to DNA-contacting residues of a set of motifs selected from the group consisting of: mQSHR-mRDHT-mRSNR; mQSHT-mRSHR-mPDHT; mQSHR-mRDHT-mRSHR; mRSHR-mRDHT-mVSNV; mQSHV-mRDHR-mRDHT; mRDER-mQSSR-mQSHT-mRSNR; mDSAR-mRSNR-mRDHT-mVSSR; mQSHT-mDSAR-mRSNR-mRDHT; mRDHT-mVSNV-mQSHT-mDSAR; mRSHR-mDSCR-mQSHT-mDSCR; mQSNR-mQSHR-mRDHT-mRSNR; mCSNR-mRDHT-mRSNR-mRSHR; mRSHR-mQSHT-mRSHR-mRDER; mQSNR-mRSHR-mQSSR-mRSHR; mQSHT-mDSCR-mRDHT-mCSNR; mQSHT-mWSNR-mRSHR-mWSNR; and mVSNV-mRSHR-mRDER-mQSNV

8. The chimeric protein of claim 1, further comprising a nuclear localization signal.

9. The chimeric protein of claim 1, wherein at least one of the zinc finger domains of the plurality has the sequence of a naturally occurring zinc finger domain.

10. The chimeric protein of claim 9, wherein at least one of the zinc finger domains of the plurality is human.

11. The chimeric protein of claim 1, wherein the protein transduction domain comprises a viral sequence or a human sequence.

12. The chimeric protein of claim 1, wherein the protein transduction domain comprises an HIV tat protein transduction domain, HSV VP22 protein, or Antennapedia homeodomain.

13. The chimeric protein of claim 12, wherein the HIV tat protein transduction domain comprises the amino acid sequence: YGRKKRRQRRR (SEQ ID NO: 1).

14. The chimeric protein of claim 1, wherein the protein transduction domain comprises a modified or synthetic protein transduction domain.

15. The chimeric protein of claim 1, wherein the protein transduction domain comprises a modified tat protein transduction domain having the amino acid sequence of any one of SEQ ID NOs: 69 to 72, a polyarginine oligopeptide consisting of 6 to 12 arginine residues, or HN1 synthetic peptide having the amino acid sequence of SEQ ID NO: 4.

16. The chimeric protein of claim 1, wherein the chimeric protein can regulate at least one endogenous gene in a cell after the protein is contacted with a membrane of the cell.

17. The chimeric protein of claim 1, wherein the chimeric protein can regulate transcription of at least one endogenous gene in a cell, but fewer than 1% of the genes in the cell.

18. The chimeric protein of claim 1, wherein the chimeric protein can regulate transcription of at least one endogenous gene in a cell, but fewer than 0.01% of the genes in the cell.

19. The chimeric protein of claim 1, wherein the chimeric protein can translocate from the extracellular milieu into a mammalian cell in the absence of a cell permeabilization reagent.

5 20. The chimeric protein of claim 1, wherein the protein transduction domain and the plurality of zinc finger domains are components of the same polypeptide chain.

21. The chimeric protein of claim 1, further comprising a cell targeting domain.

10 22. The chimeric protein of claim 21, wherein the cell targeting domain comprises an immunoglobulin variable domain, a growth factor, a cell binding domain of a viral protein, or a cell binding domain of an extracellular protein.

15 23. The chimeric protein of claim 1, further comprising a cell surface protein binding domain.

24. The chimeric protein of claim 1, further comprising a purification handle.

20 25. The chimeric protein of claim 24, wherein the purification handle comprises an amino acid sequence that can chelate metal.

26. The chimeric protein of claim 1, wherein each zinc finger domain of the plurality is bound to a zinc atom.

25 27. The chimeric protein of claim 1, further comprising a transcription activation domain.

28. The chimeric protein of claim 27, wherein the transcription activation domain comprises p65 or VP16 activation domain.

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29. The chimeric protein of claim 1, further comprising a transcription repression domain.

30. The chimeric protein of claim 29, wherein the transcription repression domain comprises Kid or KOX repression domain.

31. The chimeric protein of claim 1, wherein the protein can be transduced into at least 50% of cultured human embryonic kidney (HEK) 293 cells in an assay in which the cells are at 3×10^5 cells/ml and the protein is present in the extracellular medium at concentration of 100 micrograms/ml.

32. The chimeric protein of claim 1, wherein the chimeric protein is stable for at least 0.5 hours in human tissue culture cells.

33. A pharmaceutical composition comprising:
the chimeric protein of claim 1;
a pharmaceutically acceptable carrier.

34. The composition of claim 33, further comprising a reducing agent in an amount effective to decrease disulfide bond formation between cysteine residues of the zinc finger domain of the protein.

35. The composition of claim 34, wherein the reducing agent comprises glutathione or DTT.

36. The composition of claim 34, wherein the protein is stable in cell culture media for at least 12 hours when the composition is combined with cell culture media.

37. The composition of claim 34, further comprising between about 1 μ M and 500 μ M zinc chloride.

38. The composition of claim 33, which is used for the treatment of a subject having or being suspected of having a neoplastic disorder, an inflammatory disorder or an angiogenesis-based disorder, and the chimeric protein further comprising an effector domain such that the chimeric protein regulates VEGF-A transcription in cells of the subject.

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39. A nucleic acid comprising a coding sequence that encodes a polypeptide that comprises a) a zinc finger domain; and b) a heterologous protein transduction domain, located N-terminal to the zinc finger domain.

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40. A method of altering gene expression in a cell of a subject, the method comprising:

administering, to a subject, a chimeric DNA binding protein that comprises a plurality of zinc finger domains and a heterologous protein transduction domain, the DNA binding protein being able to regulate transcription of an endogenous gene in a cell of the subject.

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41. The method of claim 40, wherein the chimeric protein is administered as a first dose, and the method further comprises administering a second dose of the chimeric protein to the subject.

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42. The method of claim 41, wherein the first and second dose are separated by at least about 48 hours.

43. The method of claim 41, wherein the first dose is at least 25% less than the second dose.

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44. The method of claim 40, wherein the endogenous gene is VEGF-A.

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45. The method of claim 44, wherein the subject has or is suspected of having a neoplastic disorder, an inflammatory disorder or an angiogenesis-based disorder, and the DNA binding protein further comprises an effector domain such that the DNA binding protein regulates VEGF-A transcription in cells of the subject.

46. A method of altering gene expression in a eukaryotic culture cell, the method comprising:

5 contacting the cell with a dose of a chimeric DNA binding protein that comprises a plurality of zinc finger domains and a protein transduction domain, the protein being able to regulate transcription of an endogenous gene in a cell, wherein the dose is effective to regulate transcription of the endogenous gene for at least 48 hours.

10 47. The method of claim 46, further comprising, at least 48 hours after the contacting, contacting the cell with a second dose of the DNA binding protein.

48. The method of claim 46, wherein the endogenous gene is VEGF-A.

15 49. A eukaryotic cell that contains an exogenous polypeptide, but not a nucleic acid that encodes the exogenous polypeptide, wherein the exogenous polypeptide comprises a plurality of zinc finger domains and a protein transduction domain that is heterologous to the DNA binding domain, the exogenous polypeptide being functional to regulate transcription of a selected subset of endogenous genes in the cell for at least 12 hours after introduction of the exogenous polypeptide into the cell.

20 50. The eukaryotic cell of claim 49, wherein the plurality of zinc finger domains comprises a naturally occurring zinc finger domain.

25 51. The eukaryotic cell of claim 49, wherein the plurality of zinc finger domains comprises a human zinc finger domain.

52. The eukaryotic cell of claim 49, wherein exogenous polypeptide remains functional in the cell for at least 48 hours.

30 53. A method of preparing a transducible DNA binding polypeptide, the method comprising

providing a host cell that contains a nucleic acid comprising

1) a coding sequence that encodes a polypeptide that comprises a) a zinc finger domain; and b) a heterologous protein transduction domain, and

2) a promoter operably linked to the coding sequence;

5 expressing the nucleic acid in the host cell under conditions in which the polypeptide is synthesized; and

isolating the polypeptide from the host cell or from medium surrounding the host cell.

54. The method of claim 53, wherein the isolating comprises purifying inclusion
10 bodies.

55. The method of claim 53, wherein the isolating comprises affinity chromatography and ion exchange chromatography.

56. The method of claim 53, further comprising combining the isolated polypeptide
15 with a pharmaceutically acceptable carrier, thereby preparing a pharmaceutical composition.

57. A method of altering gene expression in a eukaryotic cell, the method comprising:

20 contacting a eukaryotic cell with a chimeric DNA binding protein that comprises a plurality of zinc finger domains and a protein transduction domain, the protein being able to regulate transcription of an endogenous gene in the cell.

58. The method of claim 57, wherein the eukaryotic cell is a mammalian cell.
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59. The method of claim 57, wherein the eukaryotic cell is a human cell.

60. The method of claim 57, wherein the cell is a culture cell.

30 61. The method of claim 57, wherein the cell is obtained from a subject or resides in a subject.

62. The method of claim 61, wherein the subject is human.

63. The method of claim 57, wherein the chimeric DNA binding protein comprises a plurality of zinc finger domains.

64. The method of claim 57, wherein the protein transduction domain comprises a viral sequence or a human sequence.

65. The method of claim 64, wherein the protein transduction domain comprises an HIV tat protein transduction domain, HSV VP22 protein, or Antennapedia homeodomain.

66. The method of claim 65, wherein the HIV tat protein transduction domain comprises the amino acid sequence: YGRKKRRQRRR (SEQ ID NO:1).

67. The method of claim 57, wherein the protein transduction domain comprises a modified or synthetic protein transduction domain.

68. The method of claim 57, wherein the protein transduction domain comprises a modified tat protein transduction domain having the amino acid sequence of any one of SEQ ID NOs: 69 to 72, a polyarginine oligopeptide consisting of 6 to 12 arginine residues, or HN1 synthetic peptide having the amino acid sequence of SEQ ID NO: 4.

69. The method of claim 57, wherein the protein transduction domain and the plurality of zinc finger domains are components of the same polypeptide chain.

70. The method of claim 57, wherein the chimeric DNA binding protein further comprises a cell targeting domain.

71. The method of claim 70, wherein the cell targeting domain comprises an immunoglobulin variable domain, a growth factor, a cell binding domain of a viral protein, or a cell binding domain of an extracellular protein.

5 72. The method of claim 57, wherein the endogenous gene is a gene encoding a protein selected from the group consisting of: jun B proto-oncogene, protein kinase C, lectin, brain-specific Na-dependent inorganic phosphate cotransporter, cellular retinoic acid-binding protein 1, cellular retinoic acid-binding protein 2, cadherin 13, H-cadherin (heart), vascular endothelial growth factor (VEGF-A), pigment epithelium-derived factor (PEDF),
 10 differentiation-related gene-1 (Drg-1), Transcription factor E2F, Early growth response-1 (EGR-1), protein tyrosine phosphatases 1B (PTP-1B), A20, Fas, melanoma differentiation associated gene-7 (MDA-7), presenilin-1 (PS-1), angiotensin converting enzyme, Angiopoietin-2, b-secretase(BACE1), mmp3, checkpoint with forkhead associated and ring finger (CHFR), peroxisome proliferator-activated receptor gamma (PPAR-gamma), TNF-
 15 related apoptosis-inducing ligand (TRAIL), Ku-80, ataxia-telangiectasia mutated (ATM), BRCA, CC-chemokine receptor 5 (CCR5), brain-derived neurotrophic factor (BDNF), tumor necrosis factor alpha-induced protein-3 (TNFAIP3) (A20), c-myc, Hypoxia-inducible factor-1 alpha (HIF-1 alpha), caspase-3, intercellular adhesion molecule type I (ICAM-1), angiotensin II receptor 1 (AT-1R), platelet-derived growth factor, insulin-like growth factor-I
 20 and -II, nerve growth factor, aFGF, bFGF, epidermal growth factor, TGF- α , TGF- β , erythropoietin, thrombopoietin, mucins, growth hormone, proinsulin, insulin A-chain, insulin B-chain, parathyroid hormone, thyroid stimulating hormone, thyroxine, follicle stimulating hormone, calcitonin, factor VIII, hematopoietic growth factor, enkephalinase, Mullerian-inhibiting substance, gonadotropin-associated peptide, tissue factor protein,
 25 inhibin, activin, interferon- α , interferon- β , interferon- γ , M-CSF, GM-CSF, G-CSF, IL-1, IL-2, IL-3, IL-4, IL-12, and IL-13.

73. The method of claim 57, wherein the chimeric DNA binding protein specifically binds to a site within 1000, 500, 300, or 100 base pairs of the transcriptional start site.

74. The method of claim 57, wherein the chimeric DNA binding protein specifically binds to a site within 1000, 500, 300, or 100 base pairs of the TATA box.

75. The method of claim 57, wherein the chimeric DNA binding protein specifically binds to a site within 20 base pairs or a site that overlaps with a site bound by a naturally occurring transcription factor in a regulatory region of the endogenous gene.

76. The method of claim 57, wherein the chimeric DNA binding protein increases expression of the endogenous gene.

77. The method of claim 57, wherein the chimeric DNA binding protein decreases expression of the endogenous gene.

78. The method of claim 57, wherein the cell is a neoplastic cell.

79. A host cell that comprises a nucleic acid comprising a coding sequence and a promoter operably linked to the coding sequence, wherein the coding sequence encodes a polypeptide that comprises a signal sequence, a plurality of zinc finger domains, and a protein transduction domain.

80. A method of altering expression of an endogenous gene, the method comprising: introducing, into one or more cells, a nucleic acid comprising a coding sequence and a promoter operably linked to the coding sequence, wherein the coding sequence encodes a chimeric that comprises a signal sequence, a plurality of zinc finger domains, and a protein transduction domain, such that the cells express the nucleic acid and produce and secrete the chimeric protein, whereby the chimeric protein can enter other cells and regulate expression of an endogenous gene in such cells.

81. The method of claim 80, wherein the cells into which the nucleic acid is introduced are in a subject.